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ANTIDIABETIC POTENTIAL OF WHOLE PLANT OF *PHYLLANTHUS NIRURI*

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ABSTRACT

The present study focused to study the antidiabetic activity of the whole plant of *Phyllanthus niruri* in validated animal models of diabetes. The crude powder, 95% ethanolic, 50% ethanolic and aqueous extracts of whole plant of *Phyllanthus niruri* leaves were orally administered to normoglycemic and streptozotocin (STZ)-induced diabetic rats. 95% ethanolic extract showed marked improvement on oral glucose tolerance post sucrose load in normal rats and significantly lowered the fasting blood glucose level of STZ-induced diabetic rats. Administration of 95% ethanolic extract showed marked antidyslipidemic effect on high fructose high fat diet fed Syrian golden hamsters. Further, the isolated fractions, the aqueous, n-butanol, chloroform, and hexane soluble fractions of 95% ethanolic extract of whole plants were investigated for their antihyperglycemic effect on STZ-induced diabetic rats. Of these, hexane fraction showed significant antihyperglycemic activity on STZ-induced diabetic rats at a single dose (100 mg/kg body weight). In a multiple dose study, hexane fraction when given to STZ-induced diabetic rats continuously for 30 days, the level of percent glycated hemoglobin (%HbA1c), blood glucose, hepatic and renal function markers were found towards normalization and improvement in serum insulin level and oral glucose tolerance test (OGTT) were also observed. The marked improvements in OGTT and serum insulin levels were also seen in neonatally STZ-induced diabetic rats. The hexane fraction effectively increased the glucose uptake by L-6 myotubes and inhibited the activity

of alpha (α)-glucosidase and aldose reductase enzymes. Therefore, the findings concludes hexane fraction of 95% ethanolic extract of whole plant of *P. niruri* with antidiabetic potential.

KEYWORDS: Antidiabetic activity, Antihyperglycemic activity, Glucose uptake, Neonatal-STZ induced diabetic rats, *Phyllanthus niruri* whole plant, L-6 myotubes.

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia i.e. persistent high blood glucose level caused due to insufficient insulin secretion or insulin action or both. DM is globally a major public health concern and represent main epidemic of the 21th century. According to International diabetic federation (IDF), diabetes has affected 382 million people in 2013 and its incidence is rapidly increasing and expected to affect about 600 million by the year 2035 with the majority of type 2 diabetes cases.[1] The currently available treatment of diabetes includes insulin therapeutics and oral antidiabetic agents, however these drugs produces number of serious adverse effects; thus, managing diabetes without any side effects is a challenge. Hence, the search for more safer, specific and valuable hypoglycemic agents has constantly been an area of important investigation. Therefore in this direction plant based therapeutic agents provide a promising and potential source in the treatment of diabetes as the natural products have safer side over synthetic drugs and have less or no side effects.

Phyllanthus niruri (family Euphorbiaceae), commonly called “Enyikwonwa” in south eastern part of Nigeria and “Bhumiamla” in India. It is widely spread in drier tropical area of Tamil Nadu, Madras, Kerala and Karnataka region of South-India. The whole plant, fresh leaves and fruits are used to treat various ailments, in Asia, Africa and South America[2] as a stomachic, antispasmodic, anti-hepatotoxic, antiviral, antibacterial, laxative, diuretic, carminative, in the management of diabetes, constipation, fever including jaundice, hepatitis B, dysentery, gonorrhea, syphilis, influenza, diarrhea.[3-9] Studies on extracts from various parts of the plant have revealed the hypoglycemic, hepatoprotective, hepatocurative, and hypolipidemic activities.[10-14] The crude extract of whole plant of *P. niruri* reported to have lipid lowering effect in triton induced and cholesterol fed hyperlipidemic rats for 30 days.[14, 15] Methanolic extract of aerial parts of *P. niruri* and aqueous extract of *P. niruri* leaves are known to have antihyperglycemic, antioxidant and antidyslipidemic activities in dose dependent manner in validated animal models of diabetes.[16-21] Thus, the present study was

under taken to investigate the antidiabetic role of crude extracts and their active fraction of whole plant of *P. niruri* against validated diabetic animal models.

MATERIALS AND METHODS

Chemicals: Cell culture media components like Dulbecco's Minimum Essential Media (DMEM) and fetal bovine serum (FBS) were procured from Gibco BRL, USA. Streptozotocin, metformin, glybenclamide, fenofibrate, antibiotics (pencillin G, streptomycin, gentamycin, amphoterecin B), phosphate buffered saline, trypsin, Ethylenediamine tetraacetic acid (EDTA), insulin, were purchased from Sigma Chemical Company, St. Louis, USA. 2-Deoxy-D-[^{3}H]-glucose (2-DG) was purchased from GE Healthcare, UK. Gum acacia, sucrose, glucose, fructose, and cholesterol, scintillation cocktail were purchased from Sisco Research Laboratory (India). The serum triglycerides and total cholesterol was measured using Dialab diagnostic kits. The blood glucose levels were measured using glucose strips which were obtained from Roche (India).

Animals: Adult male albino rats of Sprague Dawley (SD) strain (8–10 weeks of age, body weight (b.w.) 140±20 gm), 6 to 8 weeks old male Syrian golden hamsters of weighing around 100±20 gm (gram) body weight were procured from the animal colony of National Animal Laboratory Centre (NALC) of CSIR-Central Drug Research Institute, Lucknow, India. Research on these animals was conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Indian government in 1964. All the procedures were conducted in strict accordance with care and use of laboratory animals after necessary approval by Institutional Animal Ethical Committee (IAEC) vide no. 63/08/Biochem/IAEC dated. 01/12/2009 and 67/13/Biochem/IAEC dated. 12/06/2013. The animals were housed in animal housing facility in the standard condition of temperature, relative humidity, standard 12h light and dark cycle, noise level 50 decibel and ventilation of 10 to15 air changes per hour.1

Extraction and Fractionation of crude powder of whole plant of *P. niruri*: Whole plant of *Phyllanthus niruri* was purchased from the local market of Lucknow, India. The shade dried material was cut into fine pieces and powdered by a mechanical grinder, passed through 100 mesh sieve and the powder was stored in air-tight containers until used. One third of the crude powder (approximately 1.5 kg) of the whole plant of *P. niruri* was extracted with 10 volumes of 95% ethyl alcohol in percolator. This process was repeated five times and extract obtained each time were pooled, filtered and concentrated under reduced pressure at 55ºC in a

rota-vapor and the dried substance was termed as ethanolic extract. Similarly the 50% ethanolic extract and aqueous extract were prepared by extraction with 50% ethanol and water, respectively. All the extracts were also stored in air tight plastic containers until used. The part of ethanolic extract (200 g) was suspended in water (2.5 L) and successively fractionated with hexane, chloroform, and n-butanol saturated with water, The solvents were removed under reduced pressure to furnish corresponding fractions of hexane (8.1 gm), chloroform (6.5 gm), n-butanol (12 gm) and aqueous (10 gm).

Sucrose loaded normal rats: Male albino rats of SD of b.w. around 140 ± 20 g were selected and kept on 16h overnight starvation. Next morning the blood glucose level (0 min) of each animal was measured by glucometer using glucostrips. Animals showing blood glucose levels between 60 to 80 mg/dl (3.33-4.44 mM) were finally selected and divided into groups, consisting of six animals in each group. Each rat of experimental groups was given suspension of the crude powder and their extracts made in 1.0% gum acacia at a dosage of 250 mg/kg body weight and an equal amount of 1.0% gum acacia was administered in the control group, termed as sham treated control.[22] The standard antidiabetic drug, glybenclamide was used at dose of 25 mg/kg b.w. Just 30 min post administration of the test samples/vehicle an oral sucrose load of 10 gm/kg b.w. was given to each animal and the blood glucose level of each animal was again measured at 30, 60, 90 and 120 min post sucrose load. The percentage improvement on glucose tolerance post sucrose load was determined by plotting mean blood glucose level of each group against time and calculating the area under curve (AUC). Comparing the AUC of experimental group compared to sham treated control group determined the improvement on oral sucrose tolerance.

Streptozotocin-induced diabetic rats: To the 16 h overnight fasted 8–10 weeks old male albino rats of SD strain (b.w. 140±20 g), streptozotocin freshly prepared in citrate buffer (0.1 M, pH 4.5) at a dose of 60 mg/kg was orally administered intraperitoneally (i.p.) for induction of diabetes. The blood glucose of each animal was checked 72 h later by glucostrips to confirm the induction of diabetes and animals showing fasting blood glucose values between 280-450 mg/dl (15.5-25 mM) were selected. The selected diabetic rats were randomly divided into groups consisting of six animals in each group. The rats of experimental groups were orally administered the fine suspension of the desired test samples (made in 1.0% gum acacia) at 250 mg/kg (in case of extract), and 100 mg/kg (in case of fractions) of *P. niruri* whole plant. The standard antidiabetic drug i.e. Metformin was orally

administered at the dose of 100 mg/kg b.w. The blood glucose level of each animal was again checked by glucostrips at 30, 60, 90, 120, 180, 240, 300, and at 1440 min, respectively. Food was withdrawn, but the water was not withheld from the cages during the experimentation. The percentage blood glucose lowering by test samples or standard drug was determined by plotting blood glucose against time and calculating the area under curve (AUC) between 0-300 min and 0-1440 min and comparing the AUC of test samples treated/standard drug treated groups to that of sham treated control group. The average decline in experimental group AUC compared to control group was always termed as % antihyperglycemic activity.[23]

High fructose high fat diet fed male Syrian golden hamsters: Syrian Golden hamsters weighing around 110±10 gm were used. The animals were given the home made high fructose high fat diet (comprising of 60%, fructose, 14% saturated fat, 22% protein, 1.0 % salt mixture, 1.0 % minerals mixture and traces of vitamins) for 4 weeks. The animals showing serum triglyceride levels ≥ 400 mg/dL and total-cholesterol ≥250 mg/dL were considered as hyperlipidaemic.[24] The normal diet fed and hyperlipidemic hamsters were randomly divided into groups of six animals in each. The hamsters of Group I served as normal control (fed with normal diet and received vehicle 1% gum acacia), Group II served as dyslipidemic control (fed with high-fructose high-fat diet and received 1.0% gum acacia), whereas, Group III and IV served as experimental groups (also fed with high-fructose high-fat diet, received test samples i.e. 95% ethanolic extract of whole plant of *P. niruri* and standard drug, fenofibrate at the doses of 100 mg/kg b.w, respectively. Blood of each hamster was withdrawn from the retro-orbital plexus on day 28 post treatment. Serum was separated for immediate analysis of TG and TC on Dialab semi-auto analyzer.

Multiple dose effects of hexane fraction of whole plant of *P. niruri* on streptozotocin-induced diabetic rats and assessment of biochemical parameters: Streptozotocin induced diabetic rats with marked elevation in fasting blood glucose levels above 280-450 mg/dl were used for this study. These STZ-induced diabetic rats were left untreated for a period of 10 weeks to develop secondary complications in association with the chronic diabetes mellitus. After 10 weeks, the animals showing %HbA1c level above 8 on 0 day were considered for the study as diabetic rats and divided into groups of six rats in each. The vehicle, 1% gum acacia was given to the normal control and diabetic control groups, while the hexane fraction of ethanolic extract of *P. niruri* and metformin were given to STZ-induced rats at the desire

dose levels of (100 mg/kg) for a period of 30 days. The fasting blood glucose level of each rat was determined at the time of start and on day 7, 14, 21, and 28 when an oral glucose tolerance test of each rat was also performed post 3.0 gram/kg post glucose load.[23] On day 10, 20, and 30 the blood of each rat was withdrawn from the retro-orbital plexus for the estimation of serum triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) levels and serum AST, ALT, urea, uric acid, and creatinine levels using assay kits from Dialab diagnostic on a semiauto-analyser (DiaLab). The serum insulin level was assayed using an enzyme–linked immunosorbant assay kit as provided by Mercodia, Uppsala, Sweden. The %HbA1c level was estimated using clinical diagnostic kit (Haemoglobin RX DAYTONA # HA 3830).

Neonatally-streptozotocin induced diabetic rats: Two-day old pups of Sprague Dawley strain (weighing 7-10 gm), were injected 90 mg/kg STZ prepared in citrate buffer 0.1 M, *p*H 4.5. Non-diabetic control group receives only buffer i.p. and left along with their mothers for 4 weeks. The rats were separated from mothers after 4 weeks and further kept for 3 months in polypropylene cages, given pellet diet and water *ad libitum*. These animals showed signs of polydypsia, polyurea and abnormal OGTT at the end of the period. These rats were randomly divided into four groups of six animals in each. Group I (Normal control group) received 1.0% gum acacia, Group II (diabetic control group) received 1.0% gum acacia, Group III (experimental group) received test sample i.e. hexane fraction of whole plant of *P. niruri* at 100 mg/kg body weight dose and Group IV (standard group) received standard oral hypoglycemic drug metformin at 100 mg/kg body weight dose. Blood glucose, serum insulin and OGTT post glucose load of these animals were followed at weekly intervals for 28 days.[25]

Cell culture: L6 rat skeletal muscle cell lines, procured from National Center of Cell Sciences (NCCS), Pune, India were cultured in DMEM supplemented with 10% FBS, penicillin (100 units/ml), streptomycin (200 µg/ml) and gentamycin (50 µg/ml) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.[26, 27] Differentiation was induced by switching confluent cells to medium supplemented with 2% FBS. Experiments were performed in differentiated myotubes 4-6 days after seeding. The cells were maintained for another five to seven days and media were changed every 48h prior to use in experiments.

2-deoxy-D-[1-^{3}H] glucose uptake measurement by skeletal muscle cells (L6): Rat skeletal muscle cells (L6) were grown in 24 well plates ($6x10^4$ cells/well) and subjected to 2-

DG uptake as reported by Klip *et al.*,[28] Differentiated L6 mature myotubes were incubated with increasing concentrations of hexane fraction of *P. niruri* whole plant for 16 h with final 3 h in serum-deprived medium and a sub-set of cells were stimulated with 100 nM insulin for 20 min. Glucose uptake was assessed for 5 min in HEPES-buffered saline [140 mM NaCl, 20 mM HEPES, 5 mM KCl, 2.5 mM MgSO4, 1 mM $CaCl_2$ (pH 7.4)] containing 10 µM 2-DG (0.5 µCi/ml 2-[^{3}H] DG) at room temperature. After uptake period, radioactive solution was rapidly aspired, and the cell mono layers were rinsed three times with an ice-cold HEPES buffered containing 0.9% NaCl and 25mM D-glucose. The reaction was terminated by three washes with ice-cold HEPES buffer saline. Cells were collected in 0.1 N NaOH and cell-associated radioactivity was determined by liquid scintillation fluid in β-counter (Beckman Coulter, USA) and results were expressed as % increase glucose uptake as compare to controls. The assays was performed in triplicates and normalized to total protein, was expressed as fold change with respect to control. The results were expressed as % increase in glucose uptake as compared to controls. Backgrounds count per minute (CPM) were subtracted from control and experimental.

***In vitro* enzymatic assays**

α-Glucosidase inhibition assay: *α*-Glucosidase enzyme inhibition assay was done by the modified method of Pistia-Brueggeman and Hollingsworth, 2001.[29] 100 µl of the purified *α*-glucosidase (1 U/ml), was added to the assay system containing 500 µl of 50 mM phosphate buffer (pH 6.8), 100 µl of glutathione (1.0 mg/ml) and the desired concentrations of the test sample or standard inhibitor acarbose and the final volume was made to 1000 µl. Reaction was started by the addition of 100 µl of 1 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG) and terminated by addition of 500 µl of 0.1 M sodium carbonate. The IC_{50} value was defined as the concentration of α-glucosidase inhibitor to inhibit 50% of its activity under the assay conditions. The absorbance of colored p-nitrophenol liberated was read on 410 nm. One unit of enzyme activity is 1.0 µmol p-nitrophenol formed per min per mg protein.

Aldose Reductase inhibition assay: Lens AR activity was determined by the method as previously described by Hayman and Kinoshita.[30] The decrease in the absorption of NADPH at 340 nm over a 3 min period with DL ± Glyceraldehyde as a substrate was measured. The aldose reductase (AR) inhibitory activity was determination by adding of 0.7 ml of sodium phosphate buffer (67 mM, pH 6.2), 0.1 ml of NADPH (25×10^{-5} M), 0.1 ml of DL ± Glyceraldehyde (substrate, 1×10^{-3} M) and 0.1 ml of lens supernatant to a final volume of 1

ml were mixed in the sample cuvette and reading was taken against a reference cuvette containing all components except the substrate, DL±Glyceraldehyde. The reaction mixture was finally adjusted to pH-6.2. The enzymatic reaction was started on adding the substrate to the solution mixture, and the absorbance (OD) was recorded at 340 nm for 3 min at least 30 s intervals. The activity of aldose reductase was calculated and expressed as ΔO.D./min/mg protein. The test samples were solubilized in 100% DMSO to prepare stock solutions. To determine their AR inhibiting activity, the desired concentration from stock solution was added to reaction mixture and compared the inhibition with control (reference cuvette). The percentage inhibition (%) was calculated as [(ΔO.D. sample/min)/(ΔO.D. control/min)X100-100], where ΔO.D. sample/min stands for the decrease in absorbance for 3 min with test sample and ΔO.D. Control/min stands for the same, but with 1% DMSO instead of sample.

Statistical analysis: Statistical comparisons were made by Dunnett's test. All results were expressed as mean ± S.E. The results were considered significant if *P* values are 0.5 or less.

RESULTS

Effect of crude powder and extracts of whole plant of *P. niruri* on sucrose loaded normal rats

Table 1 presents the effect of crude powder, 95% ethanolic, ethanolic:aqueous and aqueous extracts on the improvement of oral glucose tolerance post sucrose loaded normal rats. It is evident from the results that crude powder and 95% ethanolic of *P. niruri* whole plant showed significant inhibition in post-prandial hyperglycemia of post sucrose loaded normal at 250 mg/kg of b.w. The crude powder of *P. niruri* whole plant showed around 15.6% improvement, on post prandial rise in hyperglycemia of post sucrose loaded normal rats. Whereas, ethanolic, ethanolic:aqueous and aqueous extracts of *P. niruri* whole plant showed significant improvement in glucose tolerance in order of 18.2%, 12.8% and 13.9% at dose i.e. 250 mg/kg. Whereas, the standard drug, glybenclamide showed around 31.3% improvement on oral glucose tolerance of post sucrose loaded normal rats at 25 mg/kg dose. The ethanolic extract of *P. niruri* whole plant showed marked improvement in oral glucose tolerance of post sucrose loaded normal rats (Table 1) (Fig.1a).

Table 1: Effect of crude powder and ethanolic, ethanolic:aqueous and aqueous extracts of of *P. niruri* whole plant and standard antidiabetic drugs on post sucrose loaded normal rats and STZ-induced diabetic rats

Treatment	Dose (mg/kg)	Oral glucose tolerance (OGTT) (AUC±SEM)	Blood glucose level (AUC±SEM)	
		Sucrose loaded normal rats	STZ-induced diabetic rats	
		(0-120 min)	0-300 min	0-1440 min
Sham Control (1.0% gum acacia)	-	15050±176.4	129900±5286	646800±19380
Crude Powder	**250**	12700±96.4 (15.6)**	106800±1497 (17.7)**	510200±7704 (21.1)**
95% Ethanolic extract	**250**	12310±175.0 (18.2)**	100900±991.6 (22.3)**	518900±13690 (19.8)**
Ethanolic: aqueous extract	**250**	13120±345.8 (12.8)^{ns}	109100±2127 (16.0)*	581400±6634 (10.1)^{ns}
Aqueous extract	**250**	12960±327.3 (13.9)^{ns}	112300±1397 (13.5)^{ns}	544900±17350 (15.7)*
Glybenclamide	**25**	10340±199.0 (31.3**)	-	-
Metformin	**100**	-	84160±1238 (35.2)**	465900±9023 (27.9)**

Values are mean± S.E. of six rats.

Statistical significance p*<0.05, p**<0.01 and ns, not significant compared to sham control.

Effect of crude powder and extracts of whole plant of *P. niruri* on streptozotocin-induced diabetic rats

The effect of crude powder of *P. niruri* whole plant and its extracts, ethanolic, ethanolic: aqueous, aqueous at the 250 mg/kg dose b.w and the standard antidiabetic drug metformin at a dose of 100 mg/kg on blood glucose profile of the STZ-induced diabetic rats has been illustrated in Table 1. It is evident from the result that crude powder showed significant lowering in fasting blood glucose level of STZ-induced diabetic rats. The average antihyperglycemic activity in crude powder was calculated to be around 17.8% and 21.1% during 0-300 min and 0-1440 min, respectively. Among the three extracts, the ethanolic extract of whole plant of *P. niruri* showed most significant lowering in blood glucose level of STZ-induced diabetic rats at the desired dose of 250 mg/kg b.w. The average antihyperglycemic activity of the ethanolic extract was calculated to be around 22.3 % during the period 0-300 min and 19.8 % during the period 0-1440 min. While the standard drug, metformin showed significant (p<0.01) decline in blood glucose level of STZ-induced

diabetic rats at 100 mg/kg b.w dose. The average antihyperglycemic activity of metformin was calculated to be around 35.2% ($p< 0.01$) during the period 0-300 min and 27.9 % ($p< 0.01$) during 0-1440 min, respectively (Fig. 1b and 1c).

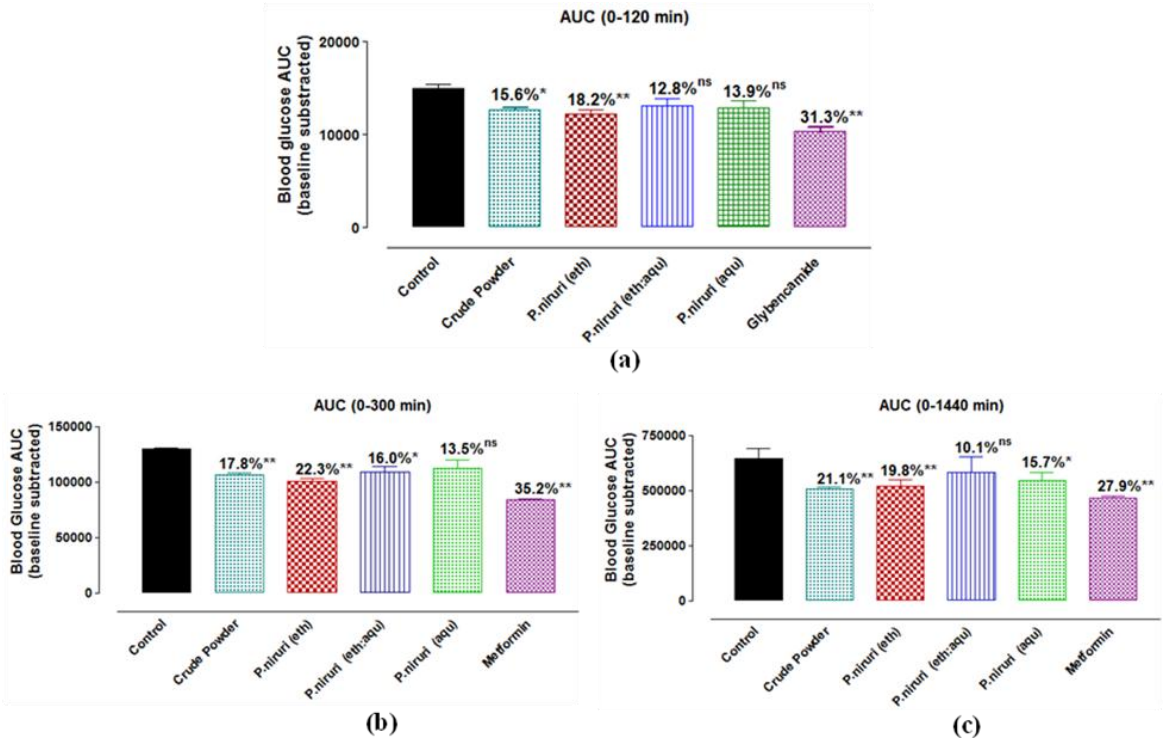

Fig.1: Effect of crude powder and ethanolic, ethanolic:aqueous and aqueous extracts of *P. niruri* whole plant and standard antidiabetic drugs (a) on post sucrose loaded normal, during 0-120 min and on STZ-induced diabetic rats (b) during 0-300 min (c) during 0-1440 min

Antidyslipidemic effect of ethanolic extract of whole plant of *P. niruri* on high fructose high fat diet fed male Syrian golden hamsters

Table 2 represents the body weight and serum lipid profile of Syrian golden hamsters treated with 95% ethanolic extract of *P. niruri* whole plant and fenofibrates for 28 consecutive days at the doses of 100 mg/kg respectively for each. Treatment with ethanolic extract of *P. niruri* whole plant to dyslipidemic hamsters caused around 14.9% and 25.5% significantly decline in their body weight while fenofibrates did not cause significant change in body weight on 14^{th} and 28^{th} day, respectively. Treatment with ethanolic extract caused dose dependent decline in their serum triglycerides by around 19.5% and 55.7%, serum cholesterol by around 18.9% and 41.2% on day 14 and 28, respectively. Whereas, the treatment of fenofibrate to these dyslipidemic hamsters caused nearly 23.3% and 57.5% decline in their serum

triglycerides, and 27.1% and 53.2% decline in their serum cholesterol on day 14 and 28, respectively.

Table 2: Effect of ethanolic extract of *P. niruri* whole plant and fenofibrate on high fructose high fat diet fed (HFHFD) Syrian hamsters

Groups	Days	Body Weight (gm)	TG (mg/dl)	TC (mg/dl)
Normal Control	**0**	133.9±3.90	243.7±13.6	151.8±11.6
	7	136.3±2.11	246.4±7.88	155.6±3.25
	14	141.4±3.58	248.1±7.88	158.2±5.02
	21	143.6±2.18	251.4±5.62	160.4±6.02
	28	147.25.03	259.7±7.86	164.2±8.23
Sham treated Dyslipidemic Control (1.0% Gum acacia)	**0**	176.1±5.36	513.7±47.3	263.2±12.7
	7	181.4±6.90	520.2±37.5	269.4±5.25
	14	185.4±5.86	528.5±20.5	273.3±12.8
	21	187.9±4.65	540.7±15.9	279.4±12.5
	28	186.7±4.20	564.1±25.7	283.4±7.91
95% Ethanolic of *P. niruri* extract treated (100 mg/kg p.o.)	**0**	170.5±4.89	513.3±35.6	264.8±11.5
	7	172.7±7.81 (4.79)^{ns}	474.4±32.3 (8.80)^{ns}	228.5±7.75 (15.1)*
	14	157.6±12.0 (14.9)*	425.3±48.6 (19.5)**	221.6±6.13 (18.9)**
	21	148.2±8.32 (21.2)**	356.4±39.4 (34.0)**	174.1±4.85 (37.7)**
	28	139.1±6.33 (25.5)**	249.7±15.0 (55.7)**	166.6±3.68 (41.2)**
Fenofibrate treated (100 mg/kg p.o.)	**0**	177.0±4.73	513.5±42.9	264.4±7.39
	7	175.4±5.78 (3.19)^{ns}	463.3±35.2 (10.9)*	219.4±6.96 (18.6)**
	14	167.8±3.69 (4.63)^{ns}	405.5±12.6 (23.3)**	199.1±6.26 (27.1)**
	21	173.7±8.29 (7.56)^{ns}	350.1±37.5 (35.3)**	170.9±4.67 (38.8)**
	28	161.9±3.41 (13.3)^{ns}	239.5±13.2 (57.5)**	132.5±4.17 (53.2)**

Values are mean ± S.E. of six hamsters, Significance: *p <0.05, **p<0.01, ns, not significant compared to sham treated control.

Effect of fractions on blood glucose levels of STZ induced diabetic rats.

Table 3, depicts the antihyperglycemic activity profile of aqueous, butanol, chloroform and hexane fractions of *P. niruri* whole pant and metformin on blood glucose level of streptozotocin induced diabetic rats at dose of 100 mg/kg. It is evident from the data in table 3 that aqueous, chloroform, butanol and hexane fractions of *P. niruri* whole plant caused an average blood glucose lowering around 10.7%, 11.6%, 0.67% and 19.4%, respectively during 0-300 min and

8.95%, 9.27%, 1.51% and 22.7%, respectively during 0-1440 min, on blood glucose levels of STZ-induced diabetic rats. Among all the fractions, maximum antihyperglycemic effect has been shown by hexane fraction of *P.niruri* whole plant which continued till 1440 min (Fig. 2). The standard antidiabetic drug, metformin caused lowering in fasting blood glucose levels of STZ-induced diabetic rats around of 26.8% (p<0.01) and 23.4% (p<0.01) during 0-300 min and 0–1440 min, respectively at 100 mg/kg of b.w. dose.

Table 3: Effect of fractions of ethanolic extract of *P. niruri* whole plant and standard drug metformin on oral glucose tolerance and blood glucose levels of STZ induced diabetic rats

Treatment	Dose (mg/kg)	Blood glucose lowering (STZ rats) (AUC±SEM)	
		0-300 min	0-1440 min
Sham treated control (1.0% Gum acacia)	**-**	119100±4874	608600±12960
Hexane fraction	**100**	**95940±3073 (19.4)****	**470400±7883 (22.7)****
Chloroform fraction	**100**	118300±4718 (0.67)^{ns}	599400±22790 (1.51)^{ns}
Butanol fraction	**100**	105300±2334 (11.6)^{ns}	552200±33900 (9.27)^{ns}
Aqueous fraction	**100**	106400±3284 (10.7)^{ns}	554100±13610 (8.95)^{ns}
Metformin treated	**100**	**87230±2991 (26.8)****	**466100±14450 (23.4)****

Values are mean± S.E. of six rats.

Statistical significance p*<0.05, p**<0.01 and ns, not significant compared to sham control.

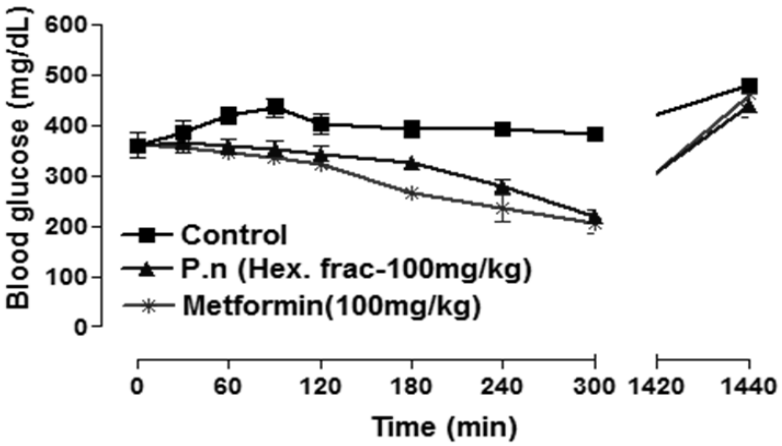

Fig 2: Effect of hexane fraction of *P. niruri* whole plant and standard antidiabetic drug, metformin on blood glucose levels of STZ induced diabetic rats.

Effect of hexane fraction of whole plant of *P. niruri* on body weight and %HbA1c of STZ induced diabetic rats

Table 4 shows the effect of hexane fraction of *P. niruri* whole plant and standard drug metformin on animal body weight and %HbA1c of STZ-induced diabetic rats. STZ induced diabetic rats showed a significant decrease in body weight compared to normal rats after 10 weeks of STZ injection. Statistically significant increase in body weight was observed after 30 days of treatment with hexane fraction of *P. niruri* and metformin treated groups. There was a significant increase in the level of glycosylated HbA1c in STZ induced diabetic rats after 10 weeks of STZ injection compared with that of normal control ($p<0.01$). Treatment with hexane fraction of *P. niruri* and metformin groups caused decline in %HbA1c level on day 30 by 37.7%, and 52.1%, respectively as compared to shame treated diabetic control (Fig. 3).

Table 4: Effect of hexane fraction of *P. niruri* whole plant and standard drug metformin on body weight and %HbA1c of STZ induced diabetic rats

Groups	Body Weight (gm)		HbA1c (%)	
	Initial (0^{th} day)	Final (30^{th} day)	Initial (0^{th} day)	Final (30^{th} day)
Normal Control (1.0% Gum acacia)	182.6±2.78	197.0±4.10	4.99±0.599	5.01±0.44
Sham treated Diabetic Control (1.0% Gum acacia)	152.6±3.46	139.8±5.03	11.9±0.84	12.4±1.14
Hexane fraction of eth.ext of *P. niruri* whole plant treated (100 mg/kg p.o)	151.0±4.15	162.6±3.90 (16.3)**	11.7±1.02	7.72±0.47 (37.7)**
Metformin treated (100 mg/kg p.o)	150.3±3.90	166.7±3.32 (19.2)**	11.9±0.98	5.94±0.59 (52.1)***

Values are mean± S.E. of six rats.

Statistical significance *$p<.05$, **$p<.01$ and ns, not significant compared to sham control

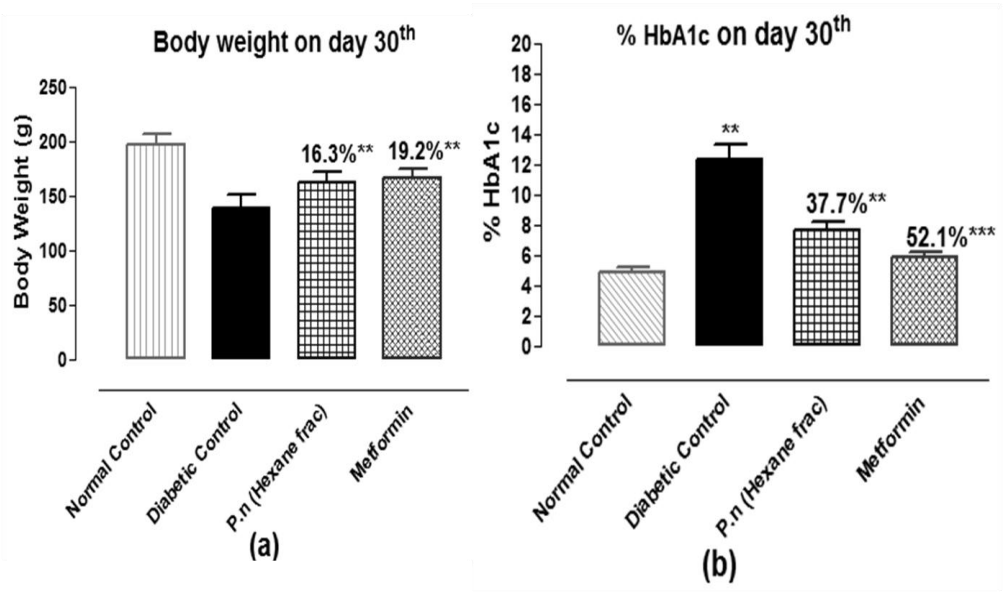

Fig 3: Effect of hexane fraction of *Phyllanthus niruri* (P.n) and metformin on (a) Body weight and (b) Serum level of % HbA1c of STZ-induced diabetic rats on day 30. Values are mean±SEM of six rats. Statistical significance *p<.05, **p<0.01, and compared to sham control.

Effects of hexane fraction of whole plant of *P. niruri* on oral glucose tolerance (OGTT) and fasting blood glucose of STZ-induced diabetic rats

Table 5 presents the effects of hexane fraction of *P. niruri* whole plant and metformin on fasting blood glucose level and on oral glucose tolerance of STZ-induced diabetic rats at the doses of 100 mg/kg for 28 consecutive days. The hexane fraction of ethanolic extract *P. niruri* whole plant and metformin treated groups showed marked significant decline in their fasting blood glucose profile from day 14 to 28, post treatment. Treatment with hexane fraction of *P. niruri* and metformin showed declined in fasting blood glucose level to the extent of 18.5% and 27.0 % and around 20.6% and 29.7 % on day 14 and 28, respectively. The hexane fraction treated group showed improvement in glucose tolerance i.e., 14.8% (p<0.05) on day 14 and around 25.0% (p <0.01) on day 28 (Fig. 4). Whereas, metformin showed improvement on OGTT around 17.2% (p<0.01) on day 14 and around 30.6% (p <0.001) on day 28, respectively, as compared to that of the sham treated control group (Fig. 4a and Fig. 4b). It is evident from the results that the effect of both on these parameters was dose dependent as the effect increased with days of treatment.

Table 5: Effects of hexane fraction of *P. niruri* whole plant and standard drug metformin on fasting blood glucose and OGTT of STZ-induced diabetic rats

Group /Treatment	Fasting blood glucose (mg/dl)					Oral Glucose tolerance (0-120 min) (AUC±SEM)				
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control (1.0% Gum acacia)	75.0± 2.76	89.0±1.99	89.4±2.78	89.9±2.78	90.5±3.01	12579±211.2	12711±152.8	12749±130.8	12800±123.8	13079±91.10
Sham treated Diabetic Control (1.0 % gum acacia)	384.2±7.09	389.2±7.13	394.0±7.57	399.7±6.73	401.8±4.92	64710±446.8	64940±138.3	64740±204.7	63880±951.9	63460±518.4
Hexane fraction of eth.ext of *P. niruri* whole plant treated (100 mg/kg p.o)	389.5±18.9	347.8±9.69 (10.6)^{ns}	321±3.99 (18.5)**	307±1.70 (23.0)**	293±8.50 (27.0)**	66350±4990	58530±610.5 (9.87)^{ns}	55130±459.6 (14.8)*	49880±821.7 (21.9)**	47570±533.2 (25.0)**
Metformin (100 mg/kg p.o)	398.0±13.3	335.0±10.3 (13.9)*	313.2±3.22 (20.6)**	301.2±6.35 (24.6)**	282.7±14.9 (29.7)**	66370±459.7	57040±457.2 (12.2)*	53600±1263 (17.2)**	48140±1381 (24.7)**	42770±4576 (30.6)**

Values are mean± S.E. of six rats.

Statistical significance p*<0.05, p**<0.01 and ns, not significant compared to sham control

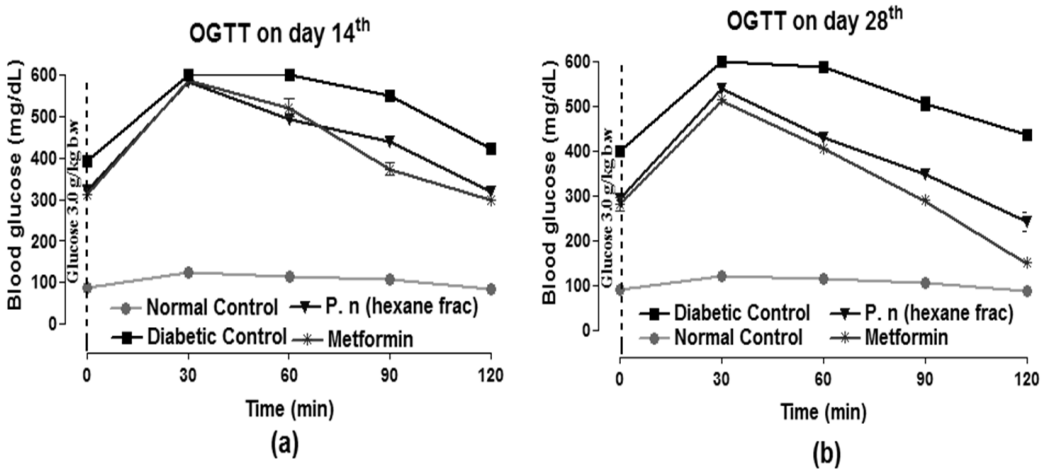

Fig. 4: Effect of hexane fraction of *P. niruri* (P. n) whole plant on glucose tolerance (OGTT) of streptozotocin induced diabetic rats (a) on day 14, and (b) on day 28.

Effect of hexane fraction of whole plant of *P. niruri* on serum insulin level and lipid profile of STZ-induced diabetic rats

Table 6 presents the effect of hexane fraction of whole plant of *P. niruri* and metformin on total insulin, triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) of STZ-induced diabetic rats. It is evident from the table 6 that the serum insulin levels in the hexane fraction of *P. niruri* and metformin treated groups were increased, i.e., in the case of a hexane fraction of *P. niruri* around 46.9% and in the case of metformin around 24.7% on day 30th post treatment. It is evident from the results as shown in table 6 that treatment with hexane fraction of ethanolic extract of *P. niruri* whole plant and metformin significantly lowered TG, TC and LDL-C and increased the HDL-C levels of STZ-induced diabetic rats. The respective lowering in these parameters was observed around 8.23, 7.04 and 9.29 on day 10 and around 27.3, 36.2 and 37.3 on day 30 in hexane fraction of ethanolic extract of *P. niruri* whole plant treated group. The metformin treated group showed lowering in these parameters around 7.72, 5.17 and 5.27 %, respectively on day 10 and around 12.8, 12.5 and 10.4, respectively on day 30. It seems that the maximum effect was achieved on day 30, in both hexane fraction of ethanolic extract of *P. niruri* whole plant as well as metformin treated group. The hexane fraction of ethanolic extract of *P. niruri* whole plant and metformin treated groups both raised serum HDL-cholesterol levels to the tune of around 11.9 and 11.7%, respectively on day 30th post treatment.1

Table 6: Effect of hexane fraction of *P. niruri* whole plant and standard drug, metformin on serum insulin level, lipid profiles of STZ-induced diabetic rats

Group/Treatment	Day	Biochemical profiles (Serum)				
		Insulin ((ng/mL)	TG (mg/dl)	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Sham treated Normal Control (1.0% Gum cacia)	**0th**	0.173±0.005	62.8±15.0	54.7±2.49	31.0±1.99	39.3±2.84
	10th	0.172±0.009	64.2±3.06	56.1±2.53	31.3±0.89	39.8±3.02
	20th	0.165±0.011	65.1±5.33	59.3±2.08	32.5±0.77	39.7±1.08
	30th	0.166±0.023	65.9±2.66	59.9±1.99	33.8±0.80	40.6±3.22
Sham treated Control (1.0% Gum cacia)	**0th**	0.086±0.001	134.7±3.12	116.3±2.35	65.7±5.09	23.7±1.66
	10th	0.084±0.001	136.0±2.58	117.8±2.00	65.6±0.80	23.0±0.80
	20th	0.082±0.003	137.5±1.61	120.8±0.99	67.2±1.01	22.8±1.60
	30th	0.081±0.003	139.0±1.44	122.3±1.33	68.0±2.17	22.5±1.86
Hexane fraction of eth. ext. of *P. niruri* whole plant treated (100 mg/kg p.o)	**0th**	0.085±0.001	131.7±3.83	118.8±3.30	68.6±7.34	23.7±1.50
	10th	0.099±0.006 (17.9)*	124.8±2.57 (8.23)^{ns}	109.5±1.08 (7.04)^{ns}	59.5±1.29 (9.29)^{ns}	23.8±1.23 (+3.60)^{ns}
	20th	0.0110±0.006 (34.7)**	115.8±2.16 (15.8)*	96.3±2.46 (19.9)**	48.0±2.86 (28.5)**	24.8±1.72 (+8.73)^{ns}
	30th	0.119±0.003 (46.9)***	101.0±1.92 (27.3)**	78.0±5.18 (36.2)**	42.7±1.22 (37.3)**	25.2±1.22 (+11.9)*
Metformin treated (100 mg/kg p.o)	**0th**	0.085±0.001	134.7±4.02	117.8±3.61	69.2±8.46	25.7±2.39
	10th	0.092±0.001 (9.52)^{ns}	125.5±2.40 (7.72)^{ns}	111.7±2.06 (5.17)^{ns}	62.2±1.23 (5.27)^{ns}	23.5±1.11 (+2.17)^{ns}
	20th	0.097±0.006 (18.3)*	124.3±1.46 (9.60)^{ns}	109.8±3.49 (8.73)^{ns}	61.7±3.06 (8.19)^{ns}	24.5±1.52 (+7.31)^{ns}
	30th	0.101±0.005 (24.7)**	121.2±2.09 (12.8) ^{ns}	107.0±3.20 (12.5)^{ns}	60.9±2.07 (10.4)^{ns}	25.0±0.94 (+11.7)^{ns}

Values are mean± S.E. of six rats.

Statistical significance p*<0.05, p**<0.01 and ns, not significant compared to sham control

Effect of hexane fraction of whole plant of *P. niruri* on liver and kidney function markers of STZ-induced diabetic rats

Table 7 presents the effect of hexane fraction of whole plant of *P. niruri* and metformin on the liver function markers i.e. AST, and ALT levels and kidney function markers, urea, uric acid, creatinine levels in serum of STZ-induced diabetic rats. Treatment with the hexane fraction of 95% ethanolic extract of *P. niruri* and metformin both, decreased the elevated levels of serum ALT and AST levels of STZ-induced diabetic rats. The respective percent decline in serum ALT and AST levels were around 10.2, and 8.62% on day 10th and around 31.9 and 28.9% on day 30th in hexane fraction of *P. niruri* treated group. The metformin treated group showed decline in these parameters to the tune of around 11.4 and 8.99% on day 10 and around 33.8 and 23.7%, respectively on day 30. The results of table 7 also reveal that both the hexane fraction and metformin decreased the elevated levels of serum urea, uric

acid and creatinine levels of STZ-induced diabetic rats. The hexane fraction of *P. niruri* and metformin both groups lowered down the levels of urea, uric acid and creatinine levels in serum at the doses of 100 mg/kg b.w. The hexane fraction of ethanolic extract of *P. niruri* treated group showed decline in the levels of urea, uric acid and creatinine in serum to around 7.74, 14.4 and 11.5 %, respectively on day 10 and the decline in the levels of these were observed nearly 20.8, 43.4 and 24.1 %, respectively on day 30, post treatment. Metformin treatment lowered serum urea level by 7.36 and 21.4%, uric acid level by 14.4 and 40.7% and serum creatinine level by 9.90 and 31.1% on day10th and 30th, respectively. It is evident from the results that there is gradual improvement over the experimental period, with the maximal beneficial effect were observed at the end of the experiment. It is evident from the results that there is gradual improvement over the experimental period, with the maximal beneficial effect were observed at the end of the experiment.

Table 7: Effect of hexane fraction of *P. niruri* whole plant and standard drug metformin on liver and kidney parameters of STZ-induced diabetic rats

Group/Treatment	Day	Biochemical Parameters (Serum)				
		Liver Function Markers		Renal Function Markers		
		ALT (U/L)	AST (U/L)	Serum-Urea (mg/dl)	Serum-Uric acid (mg/dl)	Serum-Creatinine (mg/dl)
Normal Control (1.0% Gum acacia)	**0th**	11.6±2.30	13.7±2.33	21.9±2.70	2.05±1.08	0.37±1.12
	10th	11.9±0.77	13.9±1.13	23.2±1.09	2.06±0.19	0.38±0.15
	20th	12.0±0.87	14.0±1.08	24.3±1.11	2.07±0.30	0.39±0.03
	30th	12.9±0.64	14.2±1.13	24.5±0.90	2.08±0.18	0.40±0.19
Sham treated control (1.0% Gum acacia)	**0th**	82.0±4.07	88.3±3.20	85.5±2.41	8.42±0.72	0.83±0.04
	10th	81.8±2.54	89.0±2.82	86.2±1.96	8.55±0.73	0.83±0.04
	20th	84.0±2.49	89.2±2.73	88.0±2.29	8.59±0.67	0.86±0.05
	30th	86.7±1.86	89.2±2.73	88.2±2.12	8.66±0.68	0.86±0.03
Hexane fraction of eth. ext. of *P. niruri* whole plant treated (100 mg/kg p.o)	**0th**	88.3±6.29	85.3±2.55	87.3±3.88	9.10±0.69	0.87±0.07
	10th	73.5±4.00 (10.2)^{ns}	81.3±2.56 (8.62)^{ns}	79.5±2.45 (7.74)^{ns}	7.86±0.63 (14.4)*	0.73±0.06 (11.5)^{ns}
	20th	69.3±2.86 (17.4)**	74.2±3.57 (16.8)**	77.7±2.20 (11.7)^{ns}	5.57±0.38 (35.6)**	0.72±0.03 (16.8)**
	30th	59.0±4.40 (31.9)**	63.3±3.31 (28.9)**	69.8±1.85 (20.8)**	4.90±0.39 (43.4)**	0.65±0.23 (24.1)**
Metformin Treated (100 mg/kg p.o)	**0th**	87.5±4.89	89.7±2.65	89.2±3.35	8.80±0.57	0.86±0.07
	10th	72.5±2.99 (11.4)^{ns}	81.0±4.48 (8.99)^{ns}	79.8±2.60 (7.36)^{ns}	7.32±0.54 (14.4)**	0.75±0.05 (9.90)*
	20th	61.5±2.62 (26.8)**	77.8±3.93 (12.7)*	77.0±5.02 (12.5)*	6.29±0.33 (26.7)**	0.74±0.03 (13.5)*
	30th	57.3±2.39	68.0±2.82	69.3±1.72	5.13±0.60	0.59±0.05

		(33.8)**	(23.7)**	(21.4)**	(40.7)**	(31.1)**

Values are mean± S.E. of sixrats.

Statistical significance $p^*<0.05$, $p^{**}<0.01$ and ns, not significant compared to sham control.

Effect of hexane fraction of *P. niruri* whole plant on fasting blood glucose, OGTT and serum insulin level of neonatally-STZ induced diabetic rats

Table 8 shows the effect of hexane fraction of *P. niruri* whole plant and metformin on fasting blood glucose, and oral glucose tolerance of neonatally-STZ induced diabetic rats at 100 mg/kg doses. The results clearly indicate that both the hexane fraction and metformin caused decline in fasting blood glucose level and significant improvement on OGTT and the effect was found dependent on duration of treatment. The hexane fraction of ethanolic extract of *P. niruri* whole plant treated group showed significant decline in their blood glucose profile from day 7 to day 28. The decline in fasting blood glucose levels were calculated to be around 24.9 ($p<0.01$) and 43.0% ($p<0.01$), respectively on 14 and 28 day and was comparable to the metformin treated group where around 32.3 % ($p<0.01$) and 44.7 % ($p<0.01$) lowering was observed on day 14 and 28 (Table 8). Both hexane fraction of *P. niruri* whole plant and metformin treated groups also showed improvement in their oral glucose tolerance to the tune of 21.2 and 30.5% and around 54.5 and 59.2%, respectively on day 14 and 28 (Fig. 5 a and Fig 5b).

Fig. 6 represents the effect of hexane fraction of ethanolic extract of *P. niruri* whole plant and metformin on serum insulin levels of post glucose loaded neonatally STZ-induced diabetic rats at the doses of 100 mg/kg b.w. The serum insulin levels in the hexane fraction of ethanolic extract of *P. niruri* whole plant and metformin treated groups were also increased, i.e., in the case of hexane fraction of *P. niruri* whole plant increased serum insulin level was around 43.9% and in the case of metformin increased serum insulin level was around 29.2% after 30 days of treatment.

Table 8: Effect of hexane fraction of *P. niruri* whole plant and metformin on fasting blood glucose, and OGTT of neonatally STZ–induced diabetic rats

Group/Treatment	Fasting blood glucose (mg/dl)					Oral Glucose tolerance (0-120 min) (AUC±SEM)				
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control (1.0% Gum acacia)	76.8±3.59	80.7±2.56	80.9±3.17	88.7±1.91	86.6±2.99	12439±147.2	12571±154.7	12849±129.8	12911±27.8	12912±190.2
Sham Control (1.0 % gum acacia)	191.7±3.39	190.8±3.59	191.3±3.34	192.7±2.78	193.7±2.26	46070±642.5	47090±563.9	47580±959.1	47340±1519.0	51920±1590.0
Hexane fraction of eth. ext of *P. niruri* whole plant treated (100 mg/kg p.o)	191.8±6.23	162.0±7.48 (15.1)*	143.7±2.74 (24.9)**	105.3±2.17 (45.3)**	110.3±2.86 (43.0)**	46910±1050.0	40460±1112.0 (14.0)*	37500±639.4 (21.2)**	25840±2000.0 (45.4)**	23630±382.4 (54.5)**
Metformin (100 mg/kg p.o)	191.7±15.6	164.0±2.38 (14.0)**	129.5±3.03 (32.3)**	104.7±2.24 (45.6)**	107.2±1.81 (44.7)**	46830±845.1	36900±6027 (21.6)**	33080±195.9 (30.5)**	23280±665.6 (50.8)**	21200±768.7 (59.2)**

Values are mean± S.E. of six rats.

Statistical significance $p^{*}<0.05$, $p^{**}<0.01$ and ns, not significant compared to sham control.

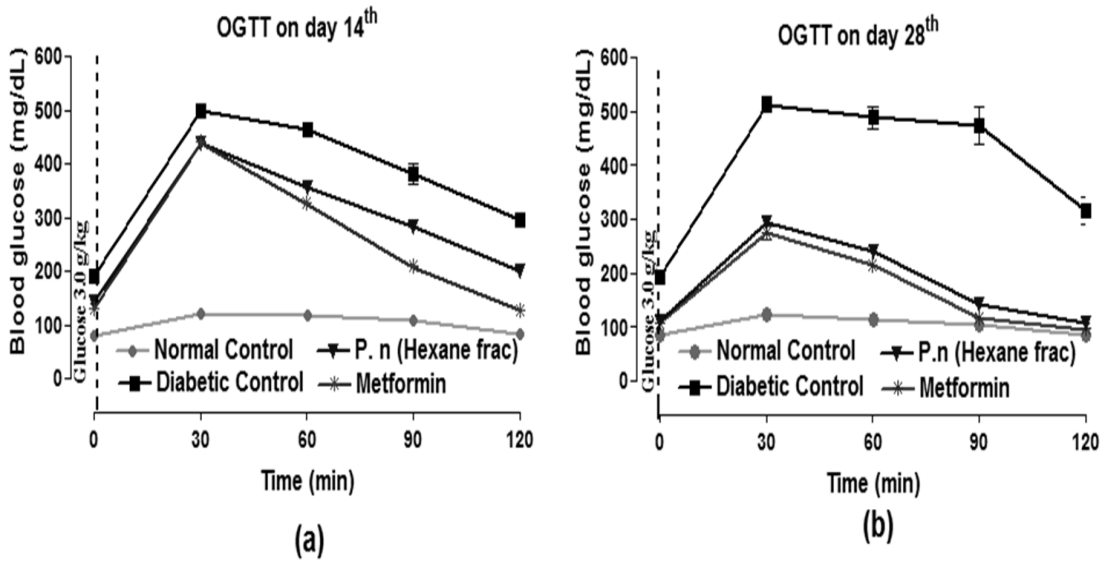

Fig. 5: Effect of hexane fraction of *P. niruri* whole plant (P. n) and metformin, on glucose tolerance of neonatally STZ treated diabetic rats (a) on day 14, and (b) on day 28.

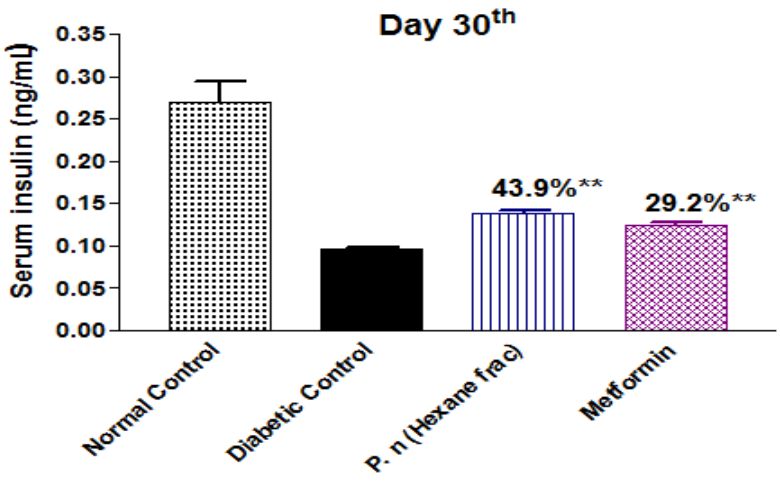

Fig. 6: Effect of Hexane fraction of ethanolic extract of *P. niruri* whole plant and metformin on serum insulin profile of neonatally STZ treated diabetic rats, Statistical significance *p<0.05, *p***<0.01.**

Effect of hexane fraction of the whole plant of *P. niruri* on glucose uptake by rat skeletal muscle cells (L6)

Fig. 7 represent the effect of hexane fractions of *P. niruri* whole plant, insulin and metformin on glucose uptake by L-6 myotubes. It is evident from the results that hexane fraction of *P. niruri* caused increases glucose uptake in concentration dependent manner in L6 cells. The hexane fraction of *P. niruri* whole plant increases basal glucose uptake in L6 myotubes to a

significant level at a minimum concentration of 10 µg/ml (1.26-fold, $p<0.05$). Maximum stimulation was observed at 20 µg/ml concentration, calculated to be around 1.52-fold ($p<0.01$) as compared to basal control cells. Results were compared with the standard antidiabetic drug Insulin and metformin. Insulin alone caused around 1.65-fold ($p<0.01$) stimulation and metformin caused nearly 1.78 fold ($p<0.01$) stimulation at 100nM and 500µM concentration, respectively (Fig. 7).

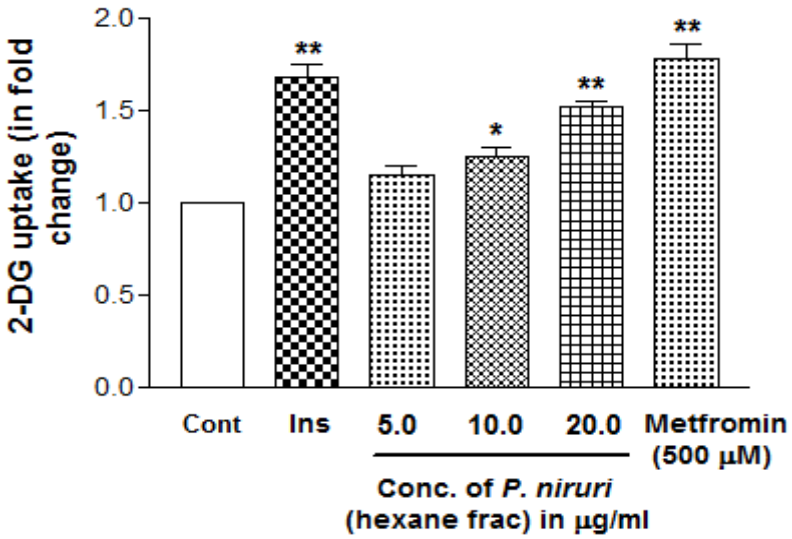

Fig. 7: Effect of hexane fractions of *P. niruri* whole plant, insulin and metformin on 2-^{3}H-deoxyglucose uptake by differentiated myotubes (L-6).

Inhibitory activity of hexane fraction of the whole plant of *P. niruri* on α-glucosidase enzyme

Fig. 8 demonstrates the effect of hexane fraction of *P. niruri* whole plant at different concentrations on α-glucosidase inhibition. *In vitro* α-glucosidase inhibition by the hexane fraction of *P. niruri* whole plant shows inhibitory potential of hexane fraction for α-glucosidase activity. The hexane fraction of *P. niruri* shows the concentration-dependent inhibition of α-glucosidase enzyme activity with around 72.4% inhibition at 100 µg/ml concentration (Fig.8). From the dose response curve, 50% inhibition (IC_{50}) value of hexane fraction of *P. niruri* whole plant was calculated to be around 50.1 µg/ml. Standard inhibitor of α-glucosidase, acarbose, at 100 µg/ml concentration exhibited around 65.4% inhibitory activity, with IC_{50} value of 49.3µg/ml under similar assay conditions.

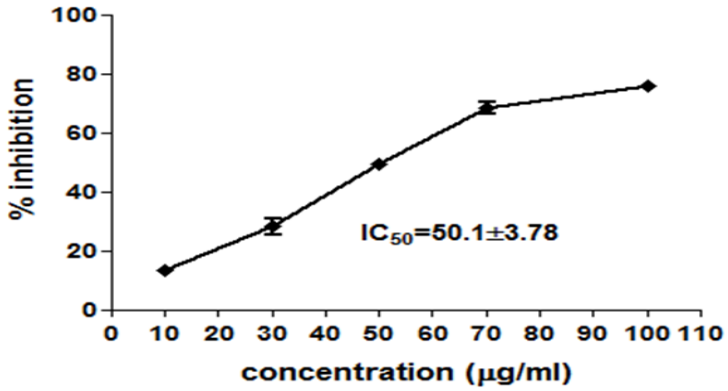

Fig. 8: The dose-response curve of *in vitro* inhibition of α-glucosidase by the hexane fraction of 95% ethanolic extract of *P. niruri* whole plant. IC_{50} Values are mean ± S.E of three independent experiments each performed in triplicate.

Inhibitory potential of hexane fraction of the whole plant of *P. niruri* on aldose reductase enzyme activity

Fig. 9 represents, the effect of hexane fraction of *P. niruri* whole plant on aldose reductase in normal and streptozotocin-induced diabetic rats. The specific activities of AR in normal and streptozotocin-induced diabetic rats were calculated to be around 0.0846 and 0.1596 µmol/min/mg protein, respectively. Incubation with hexane fraction of *P. niruri* whole plant inhibited activity of lens aldose reductase from eye lens of normal as well as STZ-induced diabetic rats in a dose-dependent manner with around 81.4% inhibition and around 96.7% inhibition at the 10 µg/ml concentration, respectively. The IC_{50} values for normal and STZ-induced diabetic rats were calculated to be around 2.74 and 1.25 µg/ml, respectively. Quercetin dehydrate, a standard inhibitor, showed IC_{50} to be around 0.69 µg/ml (2.1 µM) in normal rat eye lens and around 6.04µg/ml (20µM) in diabetic rat eye lens ((Fig. 9 a & 8b).

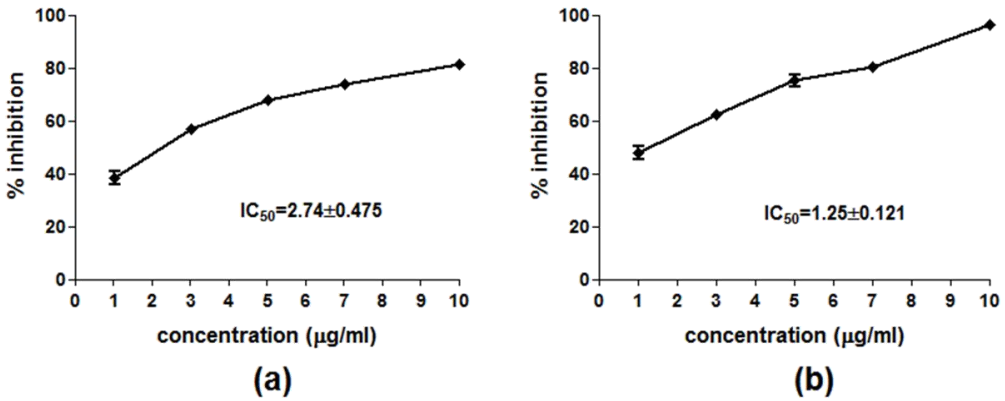

Fig. 9: Inhibition of aldose reductase *in vitro* by the hexane fraction of 95% ethanolic extract *of P. niruri* whole plant: The dose-response curve in (a) Normal eye lens (b)

Diabetic eye lens. Values are mean ± S.E of three independent experiments, each performed in triplicate.

DISCUSSION

Since the ayurveda system, the whole plant of *P. niruri* has been used as natural medicine. The present study involves the investigation of antidiabetic effect of the extracts and fractions of *P. niruri* whole plant on validated models of diabetes mellitus. The ethanolic extract of whole plant of *P. niruri* showed significant improvement in oral glucose tolerance as well as antihyperglycemic effect on sucrose loaded normal rats and STZ-induced diabetic rats.

Sucrose loaded normal rat's often referred to as physiological induction of diabetes mellitus because the blood glucose level of the animal is transiently increased with no damage to the pancreas. This model is used for Oral sucrose tolerance test (OSTT) on post sucrose loaded normal rats. In sucrose loaded normal rats in the present study, the ethanolic extract showed most significant effect as compared to ethanolic:aqueous (50% ethanolic) and aqueous extracts. The activity profiles of the study were compared to standard antidiabetic drugs i.e. glybenclamide. The standard drug glybenclamide has insulin secretaceous activity.[31, 32] It may be presumed that the crude powder, ethanolic, ethanolic: aqueous and aqueous extracts may have insulin secretaceous activity, insulin mimetic effect and might inhibits the α-glucosidase enzyme, responsible for breakdown of polysaccharides into its monomeric form and thus inhibit postprandial hyperglycemia, that lower down the blood glucose level in sucrose loaded normal rats.

The natural products and compounds are screened for their hypoglycemic/antihyperglycemic activities and insulinomimetic, insulinotropic action, by most widely used STZ-induced diabetic animal models.[33] Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea, commonly used as a diabetogenic agent.[34, 35] Nitrosourea moiety of STZ, is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across the cell membrane of beta (β) cells via glucose transporter GLUT2, causing alkylation or breakage of DNA strands and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting NAD in β-cells finally leading to energy deprivation and death of β-cells.[36, 37] It has been found in the study that the crude powder as well as ethanolic extract of *P. niruri* whole plant at the doses of 250 mg/kg of b.w caused significant decline in blood glucose level of STZ-induced diabetic rats. The

antihyperglycemic effect of crude powders as well as ethanolic, ethanolic: aqueous and aqueous extracts was also compared with that of metformin. Metformin is a biguanide already known to be an insulin sensitizer[38, 39], increases glucose utilization in the extra-hepatic tissues, and reduces hepatic gluconeogenesis.[40, 41] Blood glucose lowering effect of methanolic extract of *P. niruri* whole plant has also been reported in alloxan-induced diabetic rat model.[21]

High fat fed Syrian golden hamsters have been considered as an ideal model for studying the antidyslipidemic properties of the drugs/test samples.[42, 43] As it is evident from the results (Table 2) that after 28 days continuous feeding of the ethanolic extract of *P. niruri* whole plant at a dose of 100 mg/kg, significantly reduced triglyceride and total cholesterol levels in serum of high fructose high fat diet fed male Syrian golden hamster. The observed antidyslipidemic effect of ethanolic extract of *P. niruri* was comparable to standard antidyslipidemic drug fenofibrate at a dose of 100 mg/kg. The alcoholic extract of *P. niruri* is also reported for its lipid-lowering activity in hyperlipidaemic rats.[14]

In a single dose treatment of fractions i.e. hexane, chloroform, butanol and aqueous of ethanolic extract of *P. niruri* whole plant (100 mg/kg) were evaluated for blood glucose lowering effect on STZ-induced diabetic rats, respectively, Of all these fractions, hexane fraction showed most significant blood glucose lowering effect. The antihyperglycemic effect of the fractions was found to be comparable to standard drug metformin. Therefore, it is assumed that active fraction i.e. hexane fraction of the ethanolic extract of whole plant of *P. niruri* exhibited blood glucose lowering in the STZ-diabetic rats like metformin. The observed blood glucose lowering effect of ethanolic extract or hexane fraction of whole plant of *P. niruri* might be attributed to insulin regenerative or insulin sensitizing or insulin mimetic effect due to the phytoconstituents present in whole plant of *P. niruri.*

STZ-induced diabetic rats in the present study showed decreased body weight, elevated level of percent glycated haemoglobin, increased level of fasting blood glucose and impaired glucose tolerance as compared with the normal control rats, these results are in accordance with the previous studies. [44-46] The reduction in body weight observed in diabetic rats may be due to catabolism of fats, proteins and deficiency of carbohydrate for the energy metabolism.[47, 48] In a multiple dose experiment in the study, the oral administration of the hexane fraction of *P. niruri* whole plant at (100 mg/kg b.w dose) to STZ-induced diabetic rats for 30 consecutive days, caused a significant increase in body weight, declined percent

HbA1c levels, lowering in fasting blood glucose level and improved glucose tolerance. In the present study, after 10 weeks of the STZ injection, leads to the destruction of β-cells and decreased basal serum insulin secretion was observed.[36, 49] It is reported that diabetic complications i.e. retinopathy, nephropathy, and neuropathy are associated to increased concentration of the HbA1c levels in the blood, therefore it is utilized for diagnosis and prognosis of diabetes-associated complications.[47,50] The decrease of %HbA1c levels in diabetic rats in the study showed the potential of hexane fraction of *P. niruri* whole plant to prevent the diabetic-associated complications in STZ-induced diabetic rats. It has been observed in the study that treatment of hexane fraction of *P. niruri* whole plant exerted significantly beneficial effect by raising the serum insulin level of STZ-induced diabetic rats. This shows that the hexane fraction may stimulate the insulin release from the remaining *β*-cells or recovered pancreatic β cells.

Earlier investigations have reported the marked increased in TG, TC, and LDL-C levels and decreased HDL-C levels in serum of STZ-induced diabetic rats.[51-53] Multiple dose treatment of hexane fraction of *P. niruri* whole plant to STZ-induced diabetic rats for 30 consecutive days significantly reduced the elevated serum triglycerides, total cholesterol, and LDL-C levels, and increased the serum HDL-C level in STZ-induced diabetic rats, exhibiting potential antidyslipidemic activity of the fraction. In addition, the fraction exerted beneficial effect on improvement of altered liver and kidney function markers in STZ- induced diabetic rats as characterized by the marked lowering of the elevated serum ALT and AST levels and significantly lowering of the elevated serum urea, uric acid and creatinine levels, indicating the hepatoprotective and renoprotective effect of the hexane fraction of *P. niruri* whole plant. Our observations resemble with the earlier reports on antidiabetic, antidyslipidemic, hepatoprotective and kidney protective activities of *P. niruri*.[54, 21, 14, 3, 55, 56]

Neonatally STZ-induced diabetic rats are useful animal model of type 2 diabetes for evaluating the effect on oral glucose tolerance, β cells regeneration and insulin secretion.[57] The results of the present study showed that neonatally STZ induced diabetic rats develop moderate type 2 diabetes when compared with normal rats, however, neonatally STZ induced diabetic rats on treatment with the hexane fraction of *P. niruri* whole plant showed gradual improvement in oral glucose tolerance and significantly raised the serum insulin level in comparison to the control group, indicating the release of insulin from the regenerated

pancreatic β cells as stimulated by the hexane fraction of *P. niruri* whole plant (Table 8 and Fig. 6).

Skeletal muscle cells are considered a well-established *in vitro* model to study the regulation of glucose transport, since in skeletal muscle, glucose transporters are the first rate-limiting step for glucose utilization under physiological condition.[58] Impairment in glucose transport by transporter proteins is manifested by decreased glucose uptake is a characteristic of insulin resistance and type 2 diabetes mellitus. Thus, effect of hexane fraction of *P. niruri* whole plant on glucose utilization in mouse skeletal muscle cells has been investigated. The results of the present study indicated that incubation with a hexane fraction of the *P. niruri* significantly stimulated glucose uptake in L6 skeletal muscle cells. Incubation with 20 µg/ml concentration of the hexane fraction of *P. niruri* stimulated the glucose uptake by around 1.52-fold, which is comparable with 500 µM concentration of standard antidiabetic drug metformin which shows 1.78-fold stimulation at 20 µg/ml concentration. Since uptake of glucose is the rate limiting step in its utilization, observed antidiabetic effect of hexane fraction of *P. niruri* may be mediated, at least in part, through increased utilization of glucose in skeletal muscle which might serve as the major site for study of target of action (Fig. 7).

Postprandial hyperglycemia is an early defect of type 2 diabetes and one of primary anti-diabetic targets. Treatment of postprandial hyperglycemia can be achieved by inhibiting intestinal α-glucosidase, the key enzyme for oligosaccharide digestion and further delayed glucose absorption.[59] Thus, hexane fraction of *P. niruri* whole plant showed potential as inhibitor of α-glucosidase with IC_{50} = 50.1 µg/ml and demonstrated depressed postprandial blood glucose level (Fig. 8). AR is the first enzyme in the polyol pathway, which catalyzes the reduction of glucose to the corresponding sugar alcohol, sorbitol utilizing NADPH as a cofactor, which is subsequently metabolized to fructose by sorbitol dehydrogenase.[60, 61] Sorbitol ccumulation leads to osmotic swelling, membrane permeability changes and oxidative stress which finally cause cellular injury.[62, 63] It is evident from the results that the hexane fraction of ethanolic extract of *P. niruri* whole plant showed potential as an inhibitor for aldose reductase enzyme in eye lens of normal and diabetic rats with IC_{50} of 2.74 and 1.25 µg/ml, respectively (Fig. 9 a and Fig. 9 b). The results of the study demonstrating aldose reductase inhibitory (ARI) activity in *P. niruri* are in accordance with the previous studies.[64, 65]

CONCLUSION

In conclusion, the present study demonstrated that hexane fraction of *P. niruri* whole plant has potent antidiabetic and antidyslipidemic activity associated with an increase in glucose uptake in skeletal muscles which is a major insulin-sensitive tissue. So it might be a useful source of new antidiabetic agent for development of pharmaceutical entities or as a dietary adjunct to existing therapies of diabetes mellitus.

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